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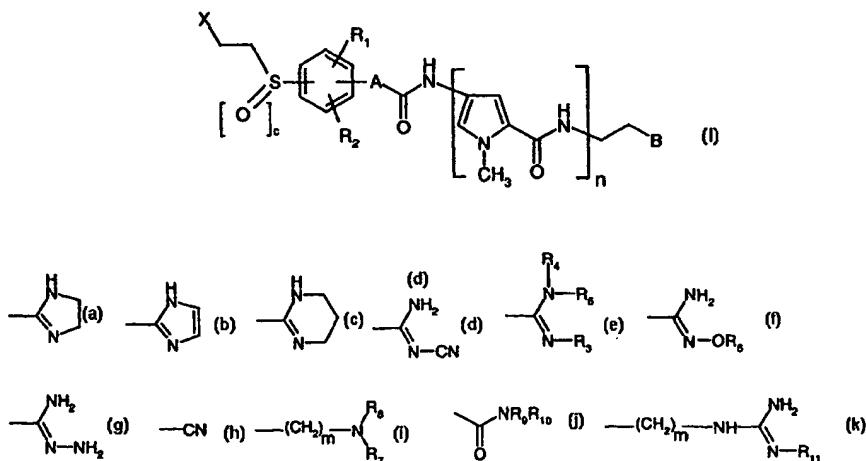
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(54) Title: OXIDISED SULFURATED DISTAMYcin DERIVATIVES, PROCESS FOR PREPARING THEM, AND THEIR USE AS ANTITUMOR AGENTS



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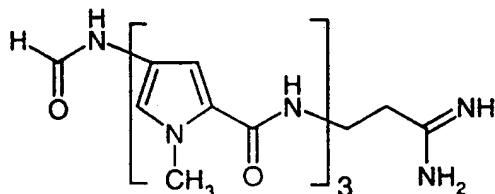
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**OXIDISED SULFURATED DISTAMYcin DERIVATIVES, PROCESS FOR
PREPARING THEM, AND THEIR USE AS ANTITUMOR AGENTS**

- 5 The present invention relates to new alkylating antitumor agents analogous to Distamycin A, to a process for their preparation, to pharmaceutical compositions containing them and to their use as therapeutic agents.

Distamycin A, whose formula is reported below



10

belongs to the family of the pyrroleamidine antibiotics and it is reported to interact reversibly and selectively with DNA-AT sequences, thus interfering with both replication and transcription. See, for a reference, Nature, 203, 1064
15 (1964); FEBS Letters, 7 (1970) 90; Prog. Nucleic Acids Res. Mol. Biol., 15, 285 (1975).

Several analogous to distamycin are known in the art. DE-A-1795539 discloses distamycin derivatives in which the formyl group is replaced by a hydrogen atom or by the
20 carboxylic acid residue of a C₁-C₄ aliphatic or cyclopentylpropionic acid.

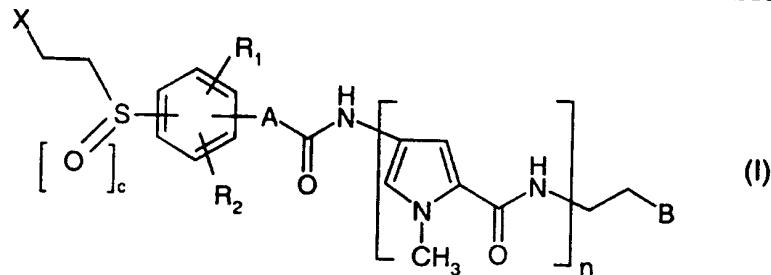
EP-A-246,868 describes distamycin analogues in which the distamycin formyl group is substituted by aromatic, alicyclic or heterocyclic moieties bearing alkylating
25 groups.

WO 97/28123 and WO 97/43258 describe distamycin analogues in which the amidino group is replaced with different nitrogen-containing ending groups and the distamycin formyl group is substituted by an aromatic or a cinnamoyl moiety,
30 respectively.

It has now been found that a new class of distamycin derivatives as defined hereinunder, wherein the distamycin formyl group is substituted by a phenylcarbonyl, phenylalkylcarbonyl or phenylalkenylcarbonyl group bearing

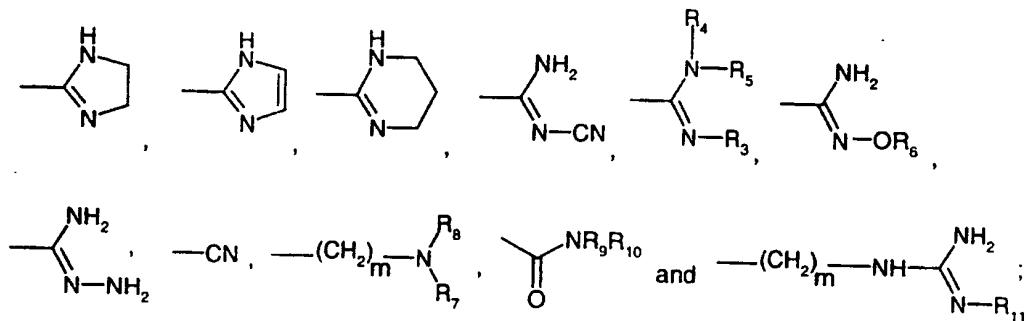
a haloethyl-sulfinyl or a haloethyl-sulfonyl group as an alkylating moiety, and the amidino group is optionally replaced by various nitrogen-containing ending groups, shows valuable biological properties.

- 5 Therefore, the present invention provides compounds which are oxidised sulfurated distamycin derivatives of formula:



wherein:

- n is 2, 3 or 4;
- 10 c is 1 or 2;
- A is a bond, a C₁-C₄ alkylene or C₂-C₄ alkenylene group; R₁ and R₂, which are the same or different, are selected from hydrogen, C₁-C₄ alkyl optionally substituted by one or more fluorine atoms, and C₁-C₄ alkoxy;
- 15 X is a halogen atom;
- B is selected from:



- wherein R₁, R₂, R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀, and R₁₁, which are the same or different, are selected from hydrogen or C₁-C₄ alkyl; R₁₁ is hydrogen, C₁-C₄ alkyl or hydroxy, and m is 0, 1 or 2; or pharmaceutically acceptable salts thereof.

The present invention includes within its scope also all the possible isomers covered by the compounds of formula (I), both separately and in admixture, as well as the metabolites and the pharmaceutically acceptable bio-

precursors (otherwise known as pro-drugs) of the compounds of formula (I).

In the present description, unless otherwise specified, both terms alkyl and alkoxy include straight or branched C₁-

5 C₄ alkyl and alkoxy groups such as, for instance, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy and tert-butoxy.

Preferred C₁-C₄ alkyl or alkoxy groups are methyl, ethyl, 10 methoxy and ethoxy groups.

When substituted by one or more fluorine atoms, the C₁-C₄ alkyl groups are preferably C₁-C₄ perfluoroalkyl groups, e.g. trifluoromethyl.

Both terms alkylene and alkenylene refer, respectively, to 15 C₁-C₄ alkylene or C₂-C₄ alkenylene groups, as bivalent radicals of the corresponding C₁-C₄ saturated or C₂-C₄ unsaturated hydrocarbons.

Preferred alkylene or alkenylene groups according to the 20 present invention are methylene, ethylene or vinylene groups.

The term halogen atom includes fluorine, chlorine, bromine and iodine, being chlorine and bromine preferred.

Within the compounds of formula (I) the haloethyl-sulfinyl 25 or sulfonyl group and the A group are in ortho, meta or para position with respect to each other; preferably, they are in meta or para position.

Pharmaceutically acceptable salts of the compounds of formula (I) are their salts with pharmaceutically acceptable either inorganic or organic acids such as, for 30 instance, hydrochloric, hydrobromic, sulfuric, nitric, acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic and p-toluenesulfonic acid.

A preferred class of compounds of the present invention is that wherein, in formula (I):

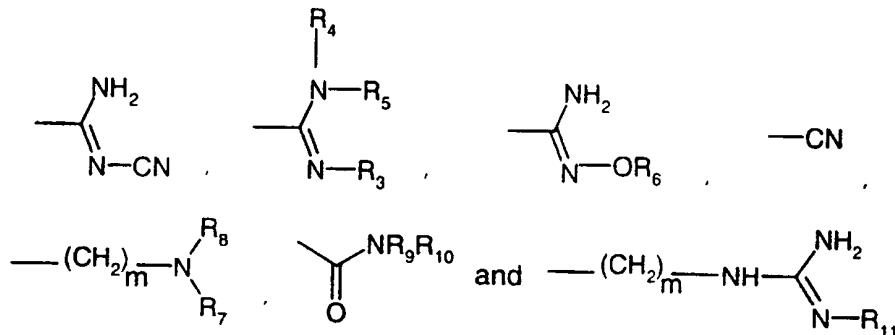
35 n is 3;

c is 1;

A is a bond or vinylene;

R_x and R_y, which are the same or different, are selected from

hydrogen, methyl, methoxy or trifluoromethyl;
 X is chloro or bromo;
 B is selected from:



5 wherein R₁, R₄, R₅, R₇, R₈, R₉, R₁₀ and R₁₁, which are the same or different, are selected from hydrogen or methyl; R₆ is hydrogen; and m is 0 or 1; or the pharmaceutically acceptable salts thereof.

10 Examples of specific compounds according to the present invention, especially in the form of salts, preferably with hydrochloric acid, are the following:

- 1) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 15 2) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;
- 20 3) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;
- 25 4) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N',N'-trimethylamidine;
- 30 5) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamidine;

- 6) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;
- 5 7) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;
- 8) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamide;
- 10 9) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;
- 15 10) 2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;
- 20 11) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N-dimethylamine;
- 25 12) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 30 13) 3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 35 14) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 15) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-

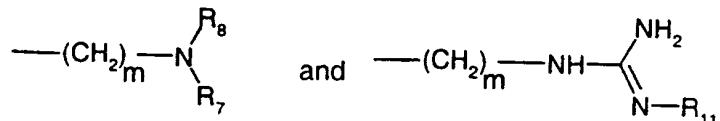
- carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-methylamidine;
- 16) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
5 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N'-dimethylamidine;
- 17) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
10 carboxamido]propion-N,N,-dimethylamidine;
- 18) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-cyanamide;
- 15) 19) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidoxime;
- 20) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamide;
- 21) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
25 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamide;
- 22) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
30 carboxamido]propionitrile;
- 23) 2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]ethylguanidine;
- 35) 24) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N,N'-trimethylamidine;

- 25) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 5 26) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamide;
- 27) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamide;
- 10 28) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamide;
- 15 29) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;
- 20 30) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;
- 25 31) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;
- 32) 2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;
- 30 33) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 35 34) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-

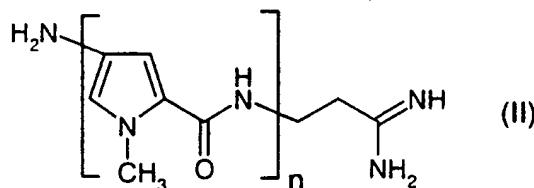
- carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-methylamidine;
- 35) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
5 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N'-dimethylamidine;
- 36) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
10 carboxamido]propion-N-cyanamide;
- 37) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidoxime;
- 15 38) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamide;
- 39) 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
20 chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionitrile;
- 40) 2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
25 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]ethylguanidine.

A further object of the present invention is a process for preparing the compounds of formula (I), and the pharmaceutically acceptable salts thereof, which process comprises:

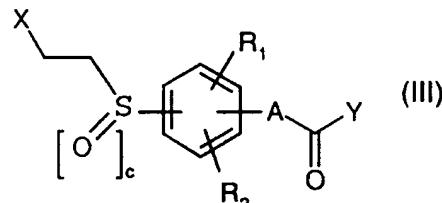
(a) when B is other than



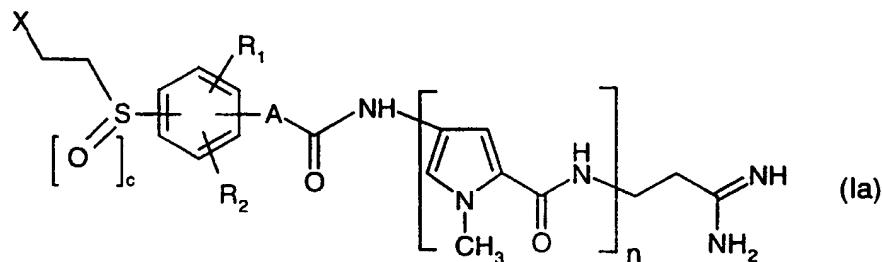
reacting a compound of formula:



with a compound of formula:

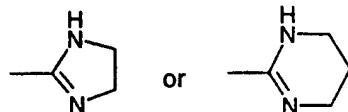


wherein n, c, R₁, R₂, X and A are as defined above, and Y is
5 hydroxy or a suitable leaving group;
so as to obtain a compound of formula:

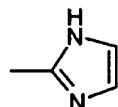


and, then, optionally reacting a compound of formula (Ia)
with:

- 10 (i) H₂N-(CH₂)_r-NH₂, wherein r is 2 or 3, so as to obtain a compound of formula (I) having B equal to:

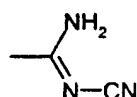


- (ii) H₂N-CH₂-CHO, so obtaining a compound of formula (I) having B equal to:

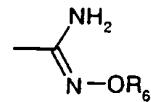


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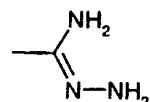
- (iii) H₂N-CN, so obtaining a compound of formula (I) having B equal to:



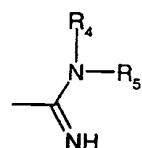
- (iv) $\text{H}_2\text{N}-\text{OR}_6$, so obtaining a compound of formula (I) having B equal to:



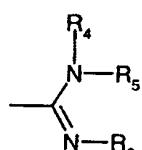
- 5 (v) $\text{H}_2\text{N}-\text{NH}_2$, so obtaining a compound of formula (I) having B equal to:



- (vi) HNR_4R_5 , so obtaining a compound of formula (I) having B equal to:



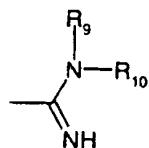
- 10 and then optionally with H_2NR_6 , so obtaining a compound of formula (I) having B equal to:



- (vii) succinic anhydride, so obtaining a compound of formula (I) having B equal to $-\text{C}\equiv\text{N}$;

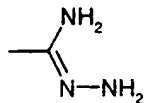
- 15 (viii) water in an alkaline medium, so obtaining a compound of formula (I) having B equal to $-\text{CONR}_9\text{R}_{10}$ wherein R₉ and R₁₀ are both hydrogen atoms;

- (ix) $\text{HNR}_9\text{R}_{10}$, so obtaining a compound of formula (I) having B equal to:

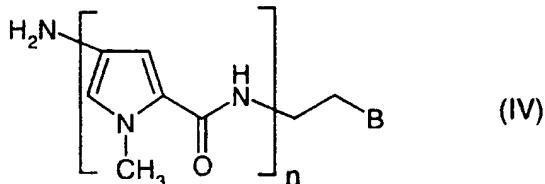


- 20 and then with water in an alkaline medium, so obtaining a compound of formula (I) having B equal to $-\text{CONR}_9\text{R}_{10}$, wherein R₉ and R₁₀ are, each independently, hydrogen or C₁-C₄ alkyl; or

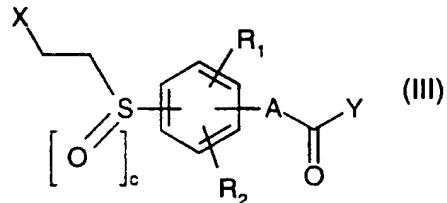
- 25 (b) when B is other than



reacting a compound of formula:



with a compound of formula:



5

wherein n, c, B, R₁, R₂, X, Y and A are as defined above; so obtaining the corresponding compound of formula (I); and, if desired, converting the compound of formula (I) into a pharmaceutically acceptable salt thereof.

- 10 In formula (III), Y is hydroxy or a leaving group selected, for instance, from chloro, 2,4,5-trichlorophenoxy, 2,4-dinitro-phenoxy, succinimido-N-oxy, imidazolyl group, and the like.

15 The condensation reactions as set forth above under processes (a) and (b) is carried out according to known methods, for instance those described in the aforementioned EP-A-246,868.

The reaction between a compound of formula (II) or (IV) with a compound of formula (III) is preferably carried out
20 with a molar ratio (II):(III) or (IV):(III) of from 1:1 to 1:2.

Within the compounds of formula (III) wherein Y is hydroxy, the reaction is carried out in an organic solvent, such as, dimethylsulphoxide, hexamethylphosphotriamide,
25 dimethylacetamide, dimethylformamide, ethanol, phenyl, or pyridine, in the presence of an organic or inorganic base such as triethylamine, diisopropyl ethylamine, or sodium or potassium carbonate or bicarbonate, and of a condensing

- agent such as, N-ethyl-N'-(3-dimethylamino-propyl)-carbodiimide, N,N'-dicyclohexyl-carbodiimide, or 1-hydroxybenzotriazole hydrate.
- The reaction temperature may vary from about -10°C to about 5 100°C, and the reaction time from about 1 to about 24 hours.
- Within the compounds of formula (III) wherein Y is a leaving group as set forth above, the aforementioned condensation reaction may be carried out in an organic 10 solvent such as, for instance, dimethylformamide, dioxane, pyridine, tetrahydrofuran, or mixtures thereof with water, optionally in the presence of an organic or inorganic base, e.g. N,N'-diisopropylethylamine, triethylamine, sodium or potassium bicarbonate, at a temperature of from about 0°C 15 to about 100°C, and for a time varying from about 2 hours to about 48 hours.
- The reaction between a compound of formula (Ia) according to process (a) and one of the reactants as described above at points (i)-(vi) or (ix), can be carried out according to 20 known methods, for instance those reported in US-4,766,142; WO 97/28123; Chem. Revs. 1961, 155; J. Med. Chem. 1984, 27, 849-857; Chem. Revs. 1970, 151; and "The Chemistry of Amidines and Imidates", edited by S. Patai, John Wiley & Sons, N.Y. (1975).
- 25 The reaction of a compound of formula (Ia) with succinic anhydride, as defined in point (vii) above, is preferably carried out with a molar ratio (Ia):succinic anhydride of from 1:1 to 1:3 in an organic solvent such as, for instance, dimethyl sulphoxide or dimethylformamide, and in 30 the presence of an organic or inorganic base such as, e.g., triethylamine, diisopropylethylamine, sodium or potassium carbonate, and the like. The reaction temperature may vary from about 25°C to about 100°C, and the reaction time from about 1 hour to about 12 hours.
- 35 The reaction with water in an alkaline medium, as defined in points (viii) and (ix) above, may be carried out according to known methods usually employed for alkaline hydrolysis, for instance by treating the substrate with an

excess of sodium or potassium hydroxide in water or in a water/organic solvent admixture, e.g. dioxane, tetrahydrofuran, or acetonitrile, at a temperature of from about 50°C to about 100°C, for a time varying from about 2 hours to about 48 hours.

The compounds of formula (II) are known or may be prepared according to known methods; see, for a reference, Arcamone et al. in *Gazzetta Chim. Ital.* 97, 1097 (1967).

Also the compounds of formula (III) are known or may be prepared according to known methods, for instance by working as described in *J. Org. Chem.* 1993, 58, 4506-4508; *Helvetica Chimica Acta*, Vol. 67, (1984), 1316-1327; *Tetrahedron Letters* 35, 3457-3460, 1994; *J. Chem. Soc. Perkin Trans. 1*, 2961, 1991.

The compounds of formula (IV) are known compounds as well, for instance as reported in the aforementioned WO 97/28123. In view of what above reported, it is clear to the man skilled in the art that when preparing the compounds of formula (I) as set forth above, optional amino groups, i.e. R₁ and/or R₂ of the compounds of formula (IV) equal to hydrogen, need to be properly protected according to conventional techniques, so as to avoid unwanted side reactions.

Likewise, the conversion of the said protected amino groups into the free amines may be carried out according to known procedures. See, for a general reference, *J. Org. Chem.* 43, 2285, (1978); *J. Org. Chem.* 44, 811 (1979); *J. Am. Chem. Soc.* 78, 1359 (1956); *Ber.* 65, 1192 (1932); and *J. Am. Chem. Soc.* 80, 1154, (1958).

Salification of a compound of formula (I), as well as preparation of a free compound starting from a salt, may be carried out by known standard methods.

Well known procedures such as, e.g., fractional crystallisation or chromatography, may also be followed for separating a mixture of isomers of formula (I) into the single isomers.

The compounds of formula (I) may be purified by conventional techniques such as, e.g., silica gel or

alumina column chromatography, and/or by recrystallisation from an organic solvent such as, e.g., a lower aliphatic alcohol, e.g. methyl, ethyl or isopropyl alcohol, or dimethylformamide.

5

PHARMACOLOGY

- The compounds of formula (I) according to the present invention are useful as antineoplastic agents.
In particular, the interest in the development of these molecules (hypoxia-selective cytotoxic agents) is related to their effect against tumor cell populations which grow at very low oxygen concentrations in solid tumors and which appear to limit the effectiveness of conventional chemotherapy.
- 15 The antineoplastic activity of the compounds was evaluated in vivo against advanced human mammary carcinoma xenograft (MX-1) showing a very good antitumor activity.
MX-1 human mammary (originally obtained from NCI, NHI, Bethesda, MD) was transplanted s.c. in athymic mice using
20 15-20 mg of tumor brei. The tumor model was maintained in vivo in adult female Hsd:athymic nude mice.
Nude mice were 4-6 weeks old, weighed 20-25 g and were maintained in cages with paper filter covers; food and bedding were sterilised and water was acidified (pH 2.5-3).
25 All animals were supplied by Harlan Nossan (Italy).
The mouse colony was routinely tested monthly for the absence of antibodies to a panel of pathogens including Mouse hepatitis, Sendai Virus and Mycoplasma pulmonis.
Drug activity was determined on advanced solid tumors (when
30 tumor mass is > 500 mg); tumor growth was assessed by caliper measurement, and tumor weight was estimated according to Geran.
The antitumor effect was determined by comparing tumor weights in the treated group and those of the control group
35 on a given day. The percentage of tumor growth inhibition (%T.I.) was calculated 7 days after the last treatment, according to the following equation:

100-(median tumor weight of treated group/median tumor weight of control group)x100

Tumor-free mice 90 days after tumor implant are considered
5 cured mice.

Toxicity was evaluated on the basis of the body weight reduction and gross autopsy findings, mainly in terms of reduction of spleen and liver size.

All drug solutions were prepared immediately before use.

10 Treatment was administered (q4dx4) intravenously in a volume of 10 ml/kg of body weight.

The compounds of the invention can be administered to mammals, including humans, through the usual routes, for
15 example, parenterally, e.g. by intravenous injection or infusion, intramuscularly, subcutaneously, topically or orally. The dosage depends on age, weight and conditions of the patient and on the administration route. For example, a suitable dosage for administration to adult humans may
20 range from about 0.1 to about 150-200 mg pro dose 1-4 times a day.

Further object of the present invention are pharmaceutical compositions, which comprise a compound of formula (I) as
25 an active principle, in association with one or more pharmaceutically acceptable carrier and/or diluent.

The pharmaceutical compositions of the present invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form. For instance, solutions for intravenous injection or infusion
30 may contain as a carrier, for example, sterile water or preferably, they may be in the form of sterile aqueous isotonic saline solutions.

Suspensions or solutions for intramuscular injections may contain, together with the active compound a
35 pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

In the forms for topical application, e.g. creams, lotions or pastes for use in dermatological treatment, the active ingredient may be mixed with conventional oleaginous or emulsifying excipients.

- 5 The solid oral forms, e.g. tablets and capsules, may contain, together with the active compound, diluents, e.g., lactose, dextrose, saccharose, cellulose, corn starch and potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols;
- 10 binding agents, e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethyl cellulose, polyvinyl-pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates, sodium starch glycolate; effervescent mixtures; dyestuffs; sweeteners; wetting agents, for
- 15 instance, lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulation. Said pharmaceutical preparations may be manufactured by known techniques, for example by means of mixing, granulating,
- 20 tabletting, sugar-coating or film-coating processes.

Further object of the present invention are the compounds of formula (I) for use in a method for treating the human or animal body by therapy.

- 25 Furthermore, the present invention provides a method for treating tumors in a patient in need of it, which comprises administering to said patient a composition of the invention.

- A further object of the present invention is a combined method for treating cancer or for ameliorating the
- 30 conditions of mammals, including humans, suffering from cancer, said method comprising administering a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumor agent, close enough in time and in amounts sufficient to produce a therapeutically useful effect.

The present invention also provides products containing a compound of formula (I), or a pharmaceutically acceptable salt thereof, and an additional antitumour agent as a

combined preparation for simultaneous, separate or sequential use in anti-cancer therapy.

The term "antitumor agent" is meant to comprise both a single antitumor drug and "cocktails" i.e. a mixture of such drugs, according to the clinical practice. Examples of antitumor agents that can be formulated with a compound of formula (I), or alternatively, can be administered in a combined method of treatment, include doxorubicin, daunomycin, epirubicin, idarubicin, etoposide, fluorouracil, melphalan, cyclophosphamide, 4-demethoxydaunorubicin, bleomycin, vinblastine, and mitomycin, or mixtures thereof.

The following examples are given to better illustrate the present invention but do not limit the scope of the invention itself.

Example 1

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine

Step I: The intermediate 4-(2-hydroxyethyl)thiobenzoic acid To a solution of 400 mg of 4-thiobenzoic acid in 2.85 ml of NaOH 2N, 0.160 ml of 2-chloroethanol were added. The solution was refluxed for 1 hour, 2.85 ml of hydrochloric acid 2N were then added dropwise and the precipitated was filtered and dried yielding 370 mg of a white solid.

FAB-MS: m/z 220, (60, [M+H]⁺)
PMR (CDCl₃) δ:

30 7.61 (d, J= 15.7 Hz, 1H), 7.33 (m, 2H), 6.55 (m, 2H), 6.21 (d, J= 15.7 Hz, 1H), 4.22 (q, J=7.1 Hz, 2H), 3.9 (b.s., 1H), 3.19 (q, J=7.1 Hz, 2H), 1.25 (t, J=7.1 Hz, 3H), 1.28 (t, J=7.1 Hz, 3H).

By analogous procedures and by using the opportune starting materials the following intermediate compounds can be obtained:

3-methyl-4(2-hydroxyethyl)thiobenzoic acid;
4-(2-hydroxyethyl)thiocinnamic acid

FAB-MS: m/z 224

PMR (DMSO-d₆) d:

7.59 (m, 2H), 7.52 (d, J = 16.0 Hz; 1H), 7.31 (m, 2H), 6.46 (d, J= 16.0 Hz, 1H), 4.9 (bs, 1H), 3.57 (t, J=6.8 Hz, 2H),
5 3.08 (t, J=6.8 Hz, 2H).

Step II: The intermediate 4-(2-chloroethyl)thiobenzoic acid

A solution of 400 mg of the intermediate, as prepared in step I, and 1.18 ml of thionyl chloride in 15 ml of toluene were refluxed for four hours, then the solvent was
10 evaporated in vacuo. The crude residue was dissolved in 20 ml acetonitrile/water (1/1) and warmed at 40°C for 1 hour. The solvent was then evaporated to dryness yielding 430 mg of a white solid which was used without further purification.

15 FAB-MS: m/z 216

PMR (CDCl₃) d:

8.01 (m, 2H); 7.38 (m, 2H); 3.67 (d, J = 7.0 Hz, 2H); 3.35 (d, J = 7.0 Hz, 2H).

By analogous procedures and by using the opportune starting
20 materials the following compound can be obtained:

3-methyl-4(2-chloroethyl)thiobenzoic acid;

Step III: The intermediate 4-(2-chloroethyl)sulfinylbenzoic acid

A solution of 430 mg of the intermediate obtained from step
25 II was added dropwise to a solution of 468 mg of NaIO₄ in 4.3 ml of water. The mixture was stirred at room temperature for 1 day, then at 80°C for 5 hours and subsequently dried under vacuum and purified by flash chromatography (Ethylacetate/Exane:8/2) to yield 320 mg of
30 the intermediate as a white solid.

By analogous procedures and by using the opportune starting materials the following compounds can be obtained:

3-methyl-4(2-chloroethyl)sulfinylbenzoic acid;

3-methyl-4(2-bromoethyl)sulfinylbenzoic acid;

35 4-(2-chloroethyl)sulfinylcinnamic acid.

Step IV: The title compound

86 mg of DCC were added to a solution of 106 mg of the intermediate obtained from step III in 4 ml of DMF and

cooled at 0°C. The solution was stirred at 0°C for 30 minutes then 200 mg of 3-[1-methyl-4-[1-methyl-4-[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine dihydrochloride (prepared as reported in J. Med. Chem 32, 774-778, 1989) and 45 mg of potassium bicarbonate were added. The solution was stirred at room temperature for 3 hours then hydrochloric acid 2N was added up to pH acid.

The solvent was then removed in vacuo and the crude residue purified by flash chromatography (methylene chloride/methanol=85/15) to yield 150 mg of the title compound as a white solid.

FAB-MS: m/z 668, (100, [M+H]⁺)

PMR (DMSO-d₆) δ:

10.56 (s, 1H), 10.00 (s, 1H), 9.92 (s, 1H), 9.0 (b.s., 2H), 8.6 (b.s., 2H), 8.21 (t, J=5.6 Hz, 1H), 8.14 (m, 2H), 7.83 (m, 2H), 7.35 (d, J=1.7 Hz, 1H), 7.24 (d, J=1.7 Hz, 1H), 7.18 (d, J=1.7 Hz, 1H), 7.12 (d, J=1.7 Hz, 1H), 7.06 (d, J=1.7 Hz, 1H), 6.94 (d, J=1.7 Hz, 1H), 4.0-3.8 (m, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.49 (m, 2H), 3.46 (m, 2H), 3.26 (m, 2H), 2.61 (t, J=6.6 Hz, 2H).

By analogous procedures and by using the opportune starting materials the following compounds can be obtained:

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N',N'-trimethylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-

carboxamido]propion-N-cyanamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-

5 carboxamido]propionamidoxime;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

10 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-

15 chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;

2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-

20 carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-

25 carboxamido]propion-N,N-dimethylamine;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-bromoethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

30 3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-

35 chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-

chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-methylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
5 chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N'-dimethylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
10 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-cyanamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
15 carboxamido]propionamidoxime;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamide;
20 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionitrile;
25 2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]ethylguanidine.

Example 2

30 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidine

Step I: The intermediate 4-(2-chloroethyl)thiocinnamic acid

35 To a solution of 150 mg of 4-(2-hydroxyethyl)thiocinnamic acid (prepared as reported in example 1 step I) in 3 ml of pyridine, 0.105 ml of mesyl chloride were added and the solution was warmed for 2 hours at 80°C. The solution was

cooled at room temperature and hydrochloric acid 37% was slowly added up to pH=1. The obtained precipitate was filtered and washed with water, then dried thus obtaining 100 mg of an orange solid.

5 FAB-MS: m/z 242

PMR (DMSO-d₆) δ:

12.3 (bs, 1H); 7.63 (m, 2H); 7.54 (d, J = 15.9 Hz, 1H); 7.34 (m, 2H); 6.48 (d, J = 15.9 Hz, 1H); 3.76 (t, J = 7.1 Hz, 2H); 3.40 (t, J = 7.1 Hz, 2H).

10 By analogous procedures and by using the opportune starting materials the following products can be obtained:

4-(2-chloroethyl)thiobenzoic acid;

4-(2-bromoethyl)thiobenzoic acid;

3-methyl-4-(2-chloroethyl)thiobenzoic acid.

15 **Step II: The intermediate 4-(2-chloroethyl)sulfinylcinnamic acid**

To 88 mg of NaIO₄ in 0.8 ml of water 90 mg of the intermediate obtained from step I, in 8 ml of MeOH, were added. The solution was warmed at 80°C for 5 hours then the 20 solvent evaporated in vacuo. The residue was chromatographed on silica gel (Ethyl acetate/Exane:7/3) yielding 45 mg of a white solid.

Step II: The title compound

A solution of 45 mg of 4-(2-chloroethyl)sulfinylcinnamic acid (prepared as described in step II), 35 mg of dicyclohexylcarbodiimide and 24 mg of 1-hydroxybenzotriazole hydrate in 3 ml of DMF, was stirred at 80°C for four hours, cooled at room temperature and then added with 90 mg 3-[1-methyl-4-[1-methyl-4-[1-methyl-4-aminopyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine dihydrochloride (prepared as reported in J. Med. Chem 32, 774-778, 1989) and 17 mg of potassium bicarbonate.

The mixture was stirred at room temperature for 2 hours, 35 the solvent was evaporated in vacuum and the crude residue purified by flash chromatography (methylene chloride/methanol: 8/2) to yield 100 mg of the title compound as a yellow solid.

FAB-MS: m/z 694, (100, [M+H]⁺)

PMR (DMSO-d₆) δ:

10.38 (s, 1H), 9.98 (s, 1H), 9.92 (s, 1H), 8.8 (b.s., 4H),
8.22 (t, J=6.0 Hz, 1H), 7.80 (m, 2H), 7.74 (m, 2H), 7.55
5 (d, J=15.6 Hz, 1H), 7.32 (d, J=1.7 Hz, 1H), 7.24 (d, J=1.7
Hz, 1H), 7.18 (d, J=1.7 Hz, 1H), 7.06 (d, J=1.7 Hz, 1H),
6.98 (d, J=1.7 Hz, 1H), 6.94 (d, J=1.7 Hz, 1H), 6.93 (d,
J=15.6 Hz, 1H), 3.86 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H),
3.76-3.96 (m, 2H), 3.48 (m, 2H), 3.2-3.45 (m, 2H), 2.61 (t,
10 J=6.5 Hz, 2H).

By analogous procedures and by using the opportune starting materials the following products can be obtained:

3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N,-dimethylamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-

carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
5 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionitrile;
2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
10 carboxamido]ethylguanidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N,N'-trimethylamidine;
15 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
20 chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-methylamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
25 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N'-dimethylamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
30 carboxamido]propion-N-cyanamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidoxime;
35 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamide;

- 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;
- 5 2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine.

10 Example 3

Tablets each weighing 0.250 g and containing 50 mg of the active substance can be manufactured as follows:

<i>Composition for 10,000 tablets</i>	
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide hydrochloride	500 g
Lactose	1,400 g
Corn starch	500 g
Talc powder	80 g
Magnesium stearate	20 g

15 The active substance, lactose and half of the corn starch were mixed; the mixture was then forced through a sieve of 0.5 mm mesh size.

20 Corn starch (10 g) was suspended in warm water (90 ml) and the resulting paste was used to granulate the powder. The granulate was dried, comminuted on a sieve of 1.4 mm mesh size, then the remaining quantity of starch, talc and magnesium stearate was added, carefully mixed and processed into tablets.

Example 4

25 Capsules, each dosed at 0.200 g and containing 20 mg of the active substance can be prepared as follows:

<i>Composition for 500 capsules</i>	
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride	10 g
Lactose	80 g
Corn starch	5 g
Magnesium stearate	5 g

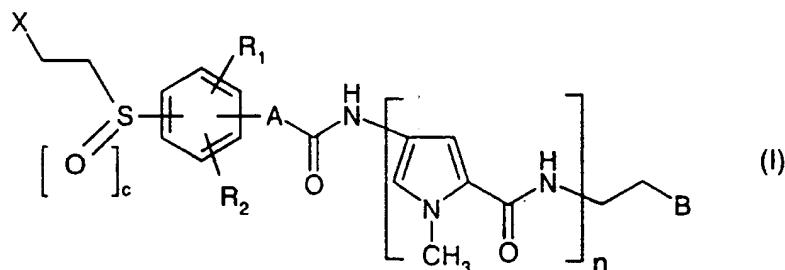
This formulation can be encapsulated in two-piece hard gelatin capsules and dosed at 0.200 g for each capsule.

Example 5

- 5 Intramuscular Injection 25 mg/ml
An injectable pharmaceutical composition can be manufactured by dissolving 25 g of 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride in sterile propylene glycol (1000 ml) and sealing ampoules of 1-5 ml.
- 10

CLAIMS

1. A compound which is an oxidised sulfurated distamycin derivative of formula:



5 wherein:

n is 2, 3 or 4;

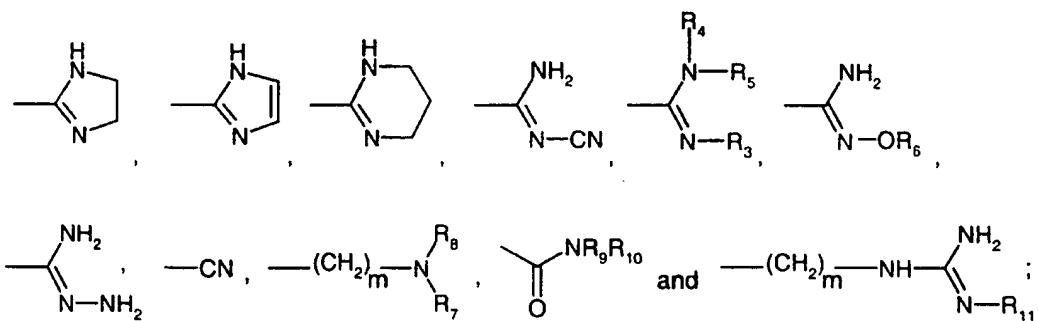
c is 1 or 2;

A is a bond, a C₁-C₄ alkylene or C₂-C₄ alkenylene group;

R₁ and R₂, which are the same or different, are selected
10 from hydrogen, C₁-C₄ alkyl optionally substituted by one or more fluorine atoms, and C₁-C₄ alkoxy;

X is a halogen atom;

B is selected from:



15

wherein R₃, R₄, R₅, R₆, R₇, R₈, R₉ and R₁₀, which are the same or different, are selected from hydrogen or C₁-C₄ alkyl; R₁₁ is hydrogen, C₁-C₄ alkyl or hydroxy, and m is 0, 1 or 2; or pharmaceutically acceptable salts thereof.

20

2. A compound according to claim 1 wherein R₁, R₄, R₅, R₆, R₇, R₈, R₉ and R₁₁ are, independently from each other, hydrogen, methyl or ethyl.

25

3. A compound according to claim 1 or 2 wherein n is 3;

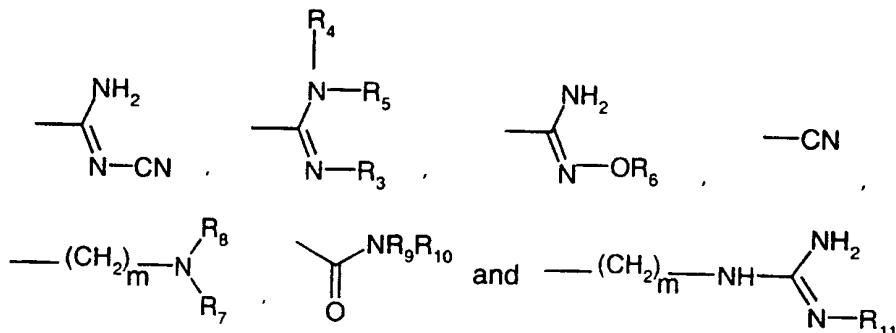
c is 1;

A is a bond or vinylene;

R₁ and R₂, which are the same or different, are selected from hydrogen, methyl, methoxy or trifluoromethyl;

5 X is chloro or bromo;

B is selected from:



wherein R₃, R₄, R₅, R₆, R₇, R₈, R₉, R₁₀ and R₁₁, which are the same or different, are selected from hydrogen or methyl; R₆ is

10 hydrogen; and m is 0 or 1;

or the pharmaceutically acceptable salts thereof.

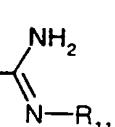
4. A compound selected from the group consisting of:

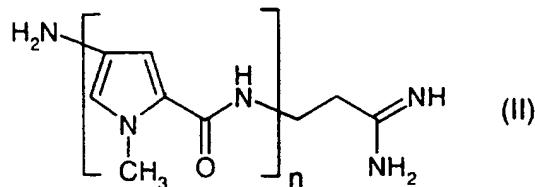
- 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;
- 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;
- 20 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;
- 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N',N'-trimethylamidine;
- 25 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
- 30

carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-cyanamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
5 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidoxime;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
10 carboxamido]propionamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-methylamide;
15 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionitrile;
2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
20 chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]ethylguanidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
25 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N-dimethylamine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
bromoethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
30 carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[3-methyl-4-(2-
chloroethylsulfinyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidine;
35 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidine;

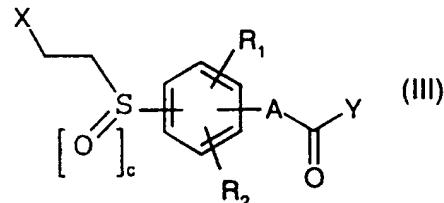
- 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;
- 5 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;
- 10 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N,-dimethylamidine;
- 15 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
- carboxamido]pyrrole-2-carboxamido]pyrrole-2-
- carboxamido]propion-N-cyanamide;
- 20 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
- carboxamido]pyrrole-2-carboxamido]pyrrole-2-
- carboxamido]propionamidoxime;
- 25 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
- carboxamido]pyrrole-2-carboxamido]pyrrole-2-
- carboxamido]propionamide;
- 30 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
- carboxamido]pyrrole-2-carboxamido]pyrrole-2-
- carboxamido]propionitrile;
- 35 2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-
- carboxamido]pyrrole-2-carboxamido]pyrrole-2-
- carboxamido]ethylguanidine;
- 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfinyl)cinnamoyl-1-carboxamido]pyrrole-2-

carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N,N'-trimethylamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
5 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
10 carboxamido]propion-N-methylamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N,N'-dimethylamidine;
15 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propion-N-cyanamidine;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
20 chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidoxime;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
25 carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamide;
3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
30 carboxamido]propionitrile;
2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)phenyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]ethylguanidine;
35 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-
chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-
carboxamido]pyrrole-2-carboxamido]pyrrole-2-
carboxamido]propionamidine;

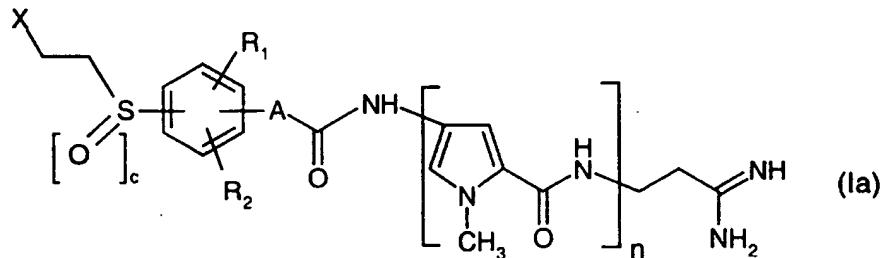
- 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-methylamidine;
- 5 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N,N'-dimethylamidine;
- 10 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propion-N-cyanamide;
- 15 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidoxime;
- 20 3-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile;
- 25 2-[1-methyl-4[1-methyl-4[1-methyl-4[4-(2-chloroethylsulfonyl)cinnamoyl-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]ethylguanidine; and the pharmaceutically acceptable salts thereof.
- 30 5. A process for preparing the compounds of formula (I), and the pharmaceutically acceptable salts thereof, which process comprises:
- (a) when B is other than
- (CH₂)_m—N^{B₈}₃₅
R₇ and —(CH₂)_m—NH—
- reacting a compound of formula:



with a compound of formula:

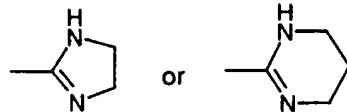


- wherein n, c, R₁, R₂, X and A are as defined in claim 1, and
 5 Y is hydroxy or a suitable leaving group;
 so as to obtain a compound of formula:

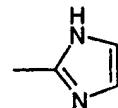


and, then, optionally reacting a compound of formula (Ia)
 with:

- 10 (i) H₂N-(CH₂)_r-NH₂, wherein r is 2 or 3, so as to obtain a
 compound of formula (I) having B equal to:

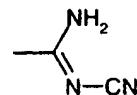


- (ii) H₂N-CH₂-CHO, so obtaining a compound of formula (I)
 having B equal to:

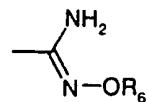


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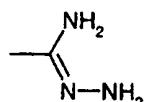
- (iii) H₂N-CN, so obtaining a compound of formula (I) having B
 equal to:



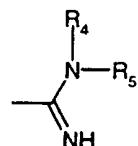
(iv) $\text{H}_2\text{N}-\text{OR}_6$, so obtaining a compound of formula (I) having B equal to:



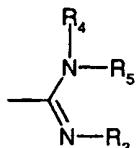
5 (v) $\text{H}_2\text{N}-\text{NH}_2$, so obtaining a compound of formula (I) having B equal to:



(vi) HNR_4R_5 , so obtaining a compound of formula (I) having B equal to:



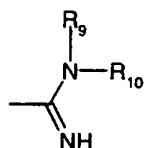
10 and then optionally with H_2NR_3 , so obtaining a compound of formula (I) having B equal to:



(vii) succinic anhydride, so obtaining a compound of formula (I) having B equal to $-\text{C}\equiv\text{N}$;

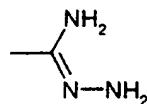
15 (viii) water in an alkaline medium, so obtaining a compound of formula (I) having B equal to $-\text{CONR}_9\text{R}_{10}$ wherein R_9 and R_{10} are both hydrogen atoms;

(ix) $\text{HNR}_9\text{R}_{10}$, so obtaining a compound of formula (I) having B equal to:

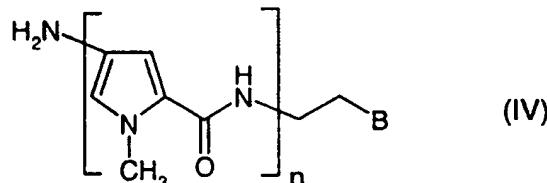


20 and then with water in an alkaline medium, so obtaining a compound of formula (I) having B equal to $-\text{CONR}_9\text{R}_{10}$, wherein R_9 and R_{10} are as defined in claim 1; or

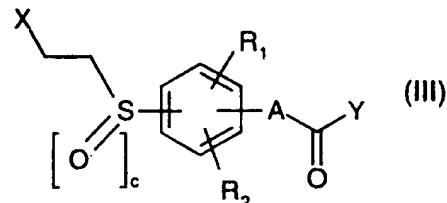
25 (b) when B is other than



reacting a compound of formula:



with a compound of formula:



5

wherein n, c, B, R₁, R₂, X, Y and A are as defined above; so obtaining the corresponding compound of formula (I); and, if desired, converting the compound of formula (I) into a pharmaceutically acceptable salt thereof.

10

6. A process according to claim 5 wherein, in the compounds of formula (III), Y is hydroxy or a group selected from chloro, 2,4,5-trichlorophenoxy, 2,4-dinitro-phenoxy, succinimido-N-oxy and imidazolyl.

15

7. A pharmaceutical composition comprising one or more pharmaceutically acceptable carriers and/or diluents and, as the active principle, a compound as defined in claim 1.

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8. A compound as defined in claim 1 for use in a method of treatment of the human or animal body by therapy.

9. A compound as defined in claim 8 for use as an antitumor agent.

25

10. Use of a compound as defined in claim 1 in the manufacture of a medicament for use as an antitumor agent.

INTERNATIONAL SEARCH REPORT

Int. Appl. No
PCT/EP 99/05349

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07D207/34 A61K31/40		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07D A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>EP 0 246 868 A (ERBA FARMITALIA) 25 November 1987 (1987-11-25) cited in the application abstract; claims 1,6-9 page 11; table page 15 -page 16; example 1 ---</p> <p>WO 97 43258 A (PHARMACIA & UPJOHN SPA ;COZZI PAOLO (IT); BERIA ITALO (IT); CALDAR) 20 November 1997 (1997-11-20) cited in the application abstract; claims 1,5-10 page 24 -page 29; example 1 ---</p> <p>-/-</p>	1,7-10 1,7-10
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.		<input checked="" type="checkbox"/> Patent family members are listed in annex.
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 13 January 2000		Date of mailing of the international search report 26/01/2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer Paisdor, B

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/EP 99/05349

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 28123 A (PHARMACIA & UPJOHN SPA ;COZZI PAOLO (IT); BERIA ITALO (IT); CALDAR) 7 August 1997 (1997-08-07) cited in the application abstract; claims 1,6-11 page 31 -page 37; example 1 -----	1,7-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern.	Final Application No
PCT/EP 99/05349	

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP 0246868	A 25-11-1987	AT 80617 T AU 597659 B AU 7316387 A BG 60531 B CA 1314551 A CS 9104137 A DE 3781716 A DK 254587 A FI 872173 A,B, GR 3006163 T HK 31993 A IE 60198 B IL 82553 A JP 1898111 C JP 6023193 B JP 62294653 A KR 9511408 B MX 9203122 A NZ 220361 A PT 84896 A,B SG 3793 G SU 1528316 A US 5017599 A US 5049579 A US 5310752 A ZA 8703593 A		15-10-1992 07-06-1990 26-11-1987 28-07-1995 16-03-1993 16-09-1992 22-10-1992 21-11-1987 21-11-1987 21-06-1993 08-04-1993 15-06-1994 10-06-1991 23-01-1995 30-03-1994 22-12-1987 04-10-1995 01-07-1992 26-04-1990 01-06-1987 12-03-1993 07-12-1989 21-05-1991 17-09-1991 10-05-1994 12-11-1987
WO 9743258	A 20-11-1997	AU 2701697 A EP 0912509 A NO 985307 A PL 329878 A		05-12-1997 06-05-1999 12-01-1999 12-04-1999
WO 9728123	A 07-08-1997	AU 1596097 A CA 2244139 A EP 0880499 A		22-08-1997 07-08-1997 02-12-1998